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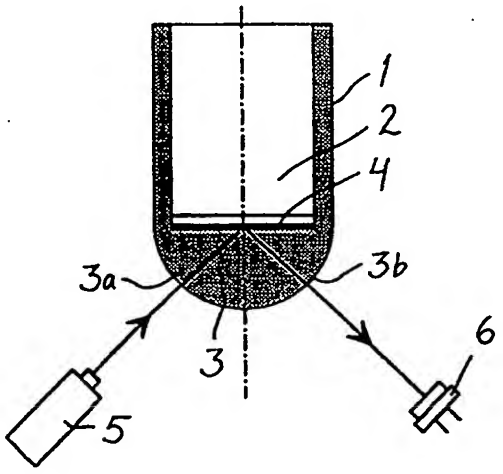
(54) Title: DEVICE FOR CARRYING OUT AN ANALYSIS

(57) Abstract

In a device for carrying out an analysis, an analysis well (1) for receiving the substance to be analyzed, a light source (5) directed towards the analysis well and a detector (6) for receiving a light beam obtained as a result of the light beam directed towards the analysis well (1) from the light source (5). The bottom of the reaction space (2) of the analysis well is coated with a layer (4) of a material capable of generating the SPR phenomenon, possibly provided with an additional coating, the bottom part of the analysis well bordering on the layer (4) is transparent to the light which together with said material generates the SPR phenomenon and is shaped into a prism (3) comprising a boundary surface (3a) for the incoming light, a boundary surface (3b) for the emerging light and a boundary surface effecting a total reflection. The light source (5) is directed through the prism (3) towards the reaction space (2) and the detector (6) is placed so as to receive the light from the prism (3). The analysis wells (1) form a multiwell structure like a strip or microtiter plate.



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Device for carrying out an analysis

The invention relates to a device for carrying out an analysis which device is of the type presented in the preamble of the enclosed claim 1.

In immunological assays analysis wells, which may be in a special microtiter plate and in which the immunological reaction is allowed to take place, are commonly used. The result is measured by means of a physical quantity obtained from the well. Examples of this kind of methods are those based on competitive binding which use fluorescence labelling and in which excitation energy in the form electromagnetic radiation of suitable wavelength is incident on the substance to be analyzed in the well and the fluorescence of the well is measured. Methods have been presented e.g. in US patents 4.374.120, 4.808.541 and 5.124.268. As to the structure of the plate and the measurement setup used in the photometric measurements reference is made to the US patent 4.648.250 showing the light source under the well and the detector above the well whereby the change in the light induced by the substance in the well is detected.

The drawback with the immunological assay methods is that to generate fluorescence one needs to use specific substances i.e. labels which are capable of generating this phenomenon. Attachment of these substances to the analyte requires an extra step.

It is known to utilize the SPR phenomenon for the determination of biochemical components present in a cuvette-like structure, e.g. in the manner presented in EP patent applications 286195 and 517930.

The purpose of the invention is to present a new kind of device for carrying out the analysis which enables one to utilize the SPR phenomenon without special reagents, yet without changing to any significant degree the structure of the analysis wells and the dosing of the substances to be analyzed into them. For accomplishing this purpose the device according to the invention is primarily characterized by what is disclosed in the characterizing portion of the enclosed claim

1. The analysis wells form a multiwell structure with at least one row of wells. The measurement based on the SPR phenomenon is conducted from the direction of the bottom of the structure through a suitable prism structure bordering on the bottom which preferably continues in the longitudinal direction if the row as a structure with a constant perpendicular cross-section. Then the detectors are also situated on the bottom side of the wells and not on the open side of the wells as in conventional measuring instruments, which utilize a through light beam, like microtiter plates, for example.

As to other advantageous embodiments of the invention reference is made to the enclosed subclaims.

Fig. 1a-e show examples of various well structures,

Fig. 2a-c show various alternatives of the measuring principle with the well of the invention,

Fig. 3 shows a combination of wells,

Fig. 4a and b show measurements with the combination of wells of Fig. 3,

Fig. 5 shows the influence of changes in the concentration to the measured values and

Fig. 6 shows the influence of biomolecules on the measured values.

Figures 1a-1e show an analysis well 1 for immunological assays which includes in a multiwell device of the invention. The result of the immunological reaction taking place in the well can be read optically based on the surface plasmon resonance technique (Surface Plasmon Resonance, SPR) known e.g. from FI patents 85766 and 84940. The well consists of an actual reaction space 2 and a prism 3. Both are made of transparent plastic, e.g polystyrene, by compressing into one

unit. A layer 4 of a material capable of generating the SPR phenomenon is attached to the inner surface of the reaction space 2 which also forms one boundary surface of the prism 3 incorporated with the well. This may be a thin film of gold evaporated or sputtered onto the surface. The outer surface of the layer 4 may be formed of a thin additional layer of dielectric material (e.g. polystyrene, glass or diamond) which protects the actual material of the layer 4 and improves attachment of a biomolecule. A biomolecule like antibody, antigen or peptide to attached to the outer surface of the material layer 4, with which the analyte molecule in the sample specifically binds. Coated in this way, the well can be stored in a dry place for a long time.

The measurement is performed so that a sample is injected into the well and it is allowed to react with an immobilized molecular layer on the surface of the layer 4. A p-polarized light beam or a luminous beam is incident through the prism 3, which forms the bottom of the well, on the material layer 4 at the boundary surface between the prism 3 and the reaction volume 2. Light is totally reflected back into the prism at the boundary surface at values of the incident angle higher than a specific limiting value of the incident angle provided that the refraction index of the material of the prism 3 is higher than that of the liquid in the reaction space 2. At a specific value of wavelength of light and incident angle, a so-called surface plasmon resonance is observed in the totally reflected light and then the reflected light disappears. In this case, all the energy of the light is transferred into electromagnetic wave propagating in the free electron plasma of the material layer 4 like gold. The resonance phenomenon amplifies the so-called evanescent electric field, which is generated in the total reflection, appearing in the reaction volume 2. This field extends only a few hundred nanometers beyond the gold surface into the reaction volume 2. The evanescent field sees the reaction taking place on the surface, e.g. the formation of a complex between the antibody bound with the surface of the material layer 4 and the antigen in the sample because it represents a definite change of the refraction index on the surface of the layer. Binding can be measured from the reflected light because resonance (disappearance of light) is shifted to another value of the incident angle.

The prism 3 forming the bottom of the analysis well 1 can be regarded as a part with a boundary surface 3a, through which the light beam enters the prism, a boundary surface, at which light is totally reflected back into the prism, and a boundary surface 3b, through which the totally reflected light beam leaves the prism. The prism 3 may have a shape of a semicylinder (Fig.1a) or a conventional triangular prism (Fig.1b) in which case its boundary surfaces 3a and 3b, through which the light beam enters and exits, come closer to each other at the bottom side of the well forming either an apex of a triangle or together a semicircle. Depending on the value of the incident angle, at which the resonance occurs, the prism may also be a part of aforesaid in which case the outer surface of the bottom of the analysis well is straight being located between the above boundary surfaces 3a, 3b, in other words, it is a kind of truncated prism (Figs. 1c and 1d). Fig.1e shows a structure of a prism in which the boundary surfaces 3a, 3b are in the middle of the prism structure coming closer to each other in the direction of the reaction volume 2, in other words, forming a kind of notch in the bottom. The incoming light beam, which is refracted at the boundary surface 3a, is reflected from the outer surface of the prism towards the layer 4 either by virtue of total reflection or by a mirror reflection effected by a reflecting surface 3c attached to the outer surface. The light totally reflected from the boundary surface which borders on the layer 4 is reflected in any of the above ways also from the second outer surface again to the second boundary surface 3b whereat it refracts out of the prism.

Figs. 2a-2c show the measuring procedure simplified. The light source 5 is a laser or a light emitting diode (LED). Reflected light can be measured with a photodiode serving as the detector 6 if only changes in the intensity of reflected light at a specific angle (Fig. 2a) are measured. Fig. 2b shows introduction of light with an optical fibre 13 in which case the luminous beam diverging from the tip of the fibre can be made collinear with a collimating optics 7. In an analogical manner, the light from the prism can be led into the detector through an optical fibre 14. If the entire resonance curve is recorded as a function of angle, as shown in Fig. 2c, a focusing optics 12 can be used between the light source 5 and the prism 3 and the diverging luminous light beam that has

undergone total reflection is measured with an CCD array detector 15 of several detectors. The luminous light beam can be collimated with optics 11, if necessary, and particularly if the array detector 15 is relatively far from the prism.

Although Figs. 2a-c show a separate optics, it is also possible to incorporate with the optics with the prism 3 in which case the lens structures are located on the surfaces 3a and 3b. The prism and the optics can then be fabricated into a unified structure e.g. by moulding.

In the structure according to the invention, several analysis wells 1 can be measured simultaneously in which case they can be arranged in a row or matrix as shown in Fig. 3. The wells can be arranged in succession in a strip 8 made of plastic in which there are several adjacent wells. These strips 8 with several wells can be further placed side by side so as to form a conventional structure of a well plate 9 or a microtiter plate. The bottoms of the strips can then have a cross-sectional shape of any of the prisms 3 shown in Figs.1a-e. Thus the bottom of the strip is formed of a continuous prism structure whose cross-section remains constant and individual prisms 3 are formed at each well 1. Figs. 1a-e can be considered to represent various possible well row or strip structures as cross-sections corresponding to the longitudinal direction of the row.

Two different methods, shown in Figs. 4a and b, can be used for reading the well plate 9:

1. The light beam from a photodiode 5 (e.g. a laser diode) is divided e.g. with a holographic element 10 and a collimator associated therewith into collinear light beams incident on each prism 3 of the well plate 9 so that the incident angle is the same in each case, Fig. 4a. Each reflected beam has its own light detector 6 measuring the changes that have taken place in the intensity.

2. The diverging light beam from the photodiode 5 is collimated into a collinear beam by an optics 7. The collinear beams are incident on

the entire area of the well plate 9. The light that has undergone total reflection with collinear beams is collected by a two dimensional CCD-detector 15 with adjacent detectors, one for each analysis well 1. Thus an image of the plate is formed in which the shift in the resonance of a specific well is seen as a change in the intensity of the light from the well, Fig. 4b.

The well plates 9 can also be read, as shown in Fig. 2c, by registering the entire resonance curve as a function of the incident angle for each well 1. The optical setup is then the Köhler illumination known per se.

When strips 8 are used, the light beams can be directed to a row of prisms 3 forming a continuous structure from e.g. a row of several light sources 5. Likewise the detectors 6 can be in a row and, when a CCD detector 15 is used, it needs to be only a one-dimensional detector. If it is desired to register the entire resonance curve as a function of the incident angle, a setup according to Fig. 2c can be used having a row of light sources 5 and a two-dimensional detector of several detectors as the CCD detector 15.

Compared to e.g. a conventional ELISA or RIA determination, the SPR cuvette and well plate presented here enables a faster determination because no labelling (additional reagents) is needed. The only additional step possibly needed is the washing of the SPR cuvette before measurement for removing the non-specifically bound material. A simple measuring procedure also enables development of cheap portable measuring instruments e.g. for environmental analytics.

The light from the light source must be p-polarized. If the light source itself does not have a polarizer, this can be realized by a separate polarizer placed between the light source and the prism shown under the reference numeral 16 in Figs. 2b, 2c, and 4b.

In the case of the strip or the well plate, multiple sample measurement as well as a positive and a negative control sample can be used for

checking the result. The strips or the plates may also have separate control coatings in a sealed space for calibrating the measurement.

The well strips and the well plates can be fabricated so that their external dimensions are the same as those of existing strips and plates. Thus new dosing and incubation equipment are not needed. The above structures can be fabricated by spray moulding using plastic material of appropriate optical quality, after which the bottoms of the wells 1 can be coated with a SPR material layer 4 using some known technique. Instead of assembling from separate strips 8, the well plate 9 can be manufactured also by moulding from a single piece thereby forming, in a corresponding fashion, prism structures which are parallel with the well rows and which have a constant cross-section.

Fig. 5 shows the influence of a NaCl dilution series 300-80 mM on the intensity of the light obtained in the analysis. As the test series shows, the concentrations of the liquid in the reaction space, which borders on the thin layer of gold, has a clear influence on the light intensity. The measurement has been conducted on the downwards sloping part of the curve representing the intensity as a function of the incident angle in which case an increase in the concentration at a specific constant value of the incident angle effecting the SPR phenomenon causes shift of the curve to the right and, at the same time, an increase in the intensity values of the light that has undergone the SPR phenomenon.

Fig. 6 shows the influence of binding of a synthetic peptide and HIV antibody on the intensity values. During the time interval A (0-750 s) buffer solution has been added to the well. At point B (750 s) rinsing and addition of the peptide (50 ug/ml) has been performed. During the time interval C (1400 - 1600 s) rinsings with the buffer solution has been performed, at point D (1600 s) antibody (1/24000 titer) has been added, and during the interval E (3400 - 3600 s) rinsings have been performed. The influence of the above actions and binding between biomolecules on the intensity values of the SPR measurement can be clearly observed and the figure is a good example how the reaction in the reaction space 2 or in several reaction spaces 2 can be continuously monitored in the device according to the invention.

Although the purpose of use of the invention is primarily the monitoring of the reactions between biomolecules and their qualitative and quantitative analysis, the invention can be applied in all kinds of analyses in which a change in the analyte in the reaction space causes a sufficiently clear change in the light that has gone through the prism.

Claims:

1. Device for carrying out an analysis having an analysis well (1) for receiving the substance to be analyzed, a light source (5) directed towards the analysis well and a detector (6) for receiving a light beam obtained as a result of the light beam directed towards the analysis well (1) from the light source (5), the bottom of the reaction space (2) of the analysis well being coated with a layer (4) of a material capable of generating the SPR phenomenon, possibly provided with an additional coating, the bottom part of the analysis well bordering on the layer (4) being transparent to the light which together with said material generates the SPR phenomenon and being shaped into a prism (3) comprising a boundary surface (3a) for the incoming light, a boundary surface (3b) for the emerging light and a boundary surface effecting a total reflection, the light source (5) being directed through the prism (3) towards the reaction space (2) and the detector (6) being placed so as to receive the light from the prism (3), **characterized** in that the analysis wells (1) form a multiwell structure (8,9) which comprises at least one row of wells separated from one another and in the bottom of which adjacent prisms (3) are located at the bottoms of the corresponding reaction spaces (2).

2. Device according to claim 1 **characterized** in that the prisms (3) form a continuous prism structure in the direction of the row of wells (1) with a constant cross-section perpendicular to the longitudinal direction of the row.

3. Device according to claim 1 or 2 **characterized** in that the structure is a strip (8) consisting of a single row of wells (1).

4. Device according to claim 1 or 2 **characterized** in that the structure is a well plate (9) with several rows of wells (1).

5. Device according to claim 4 **characterized** in that the well plate (9) consists of strips (8) placed side by side.

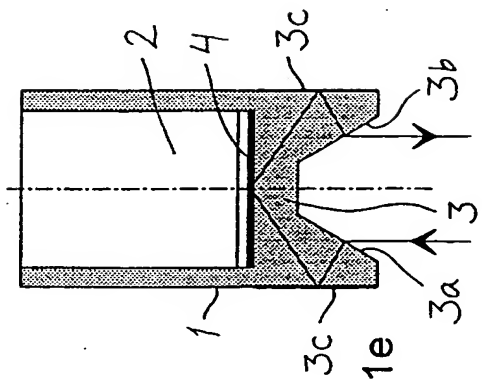

Fig. 1e

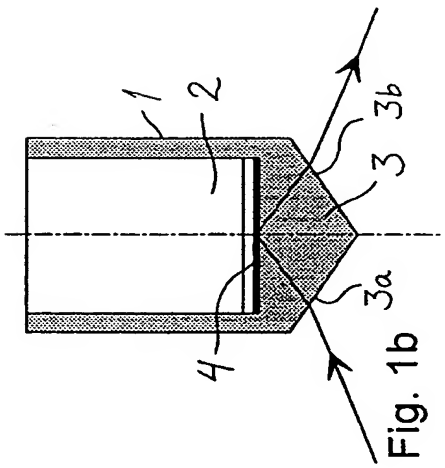

Fig. 1b

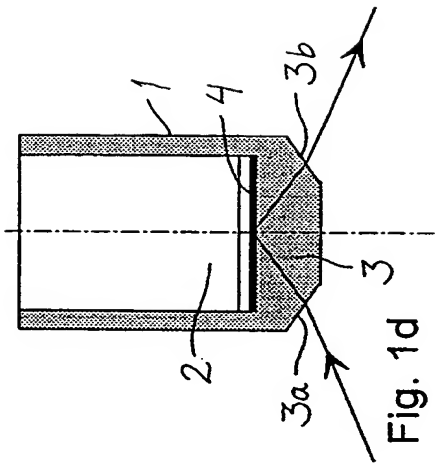

Fig. 1d

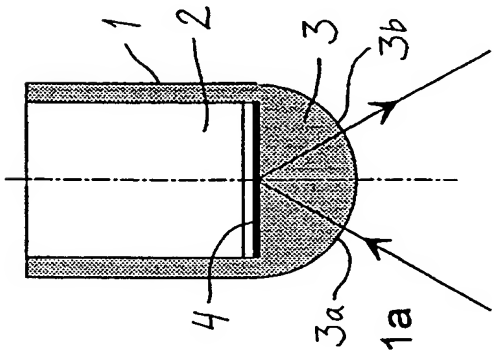

Fig. 1a

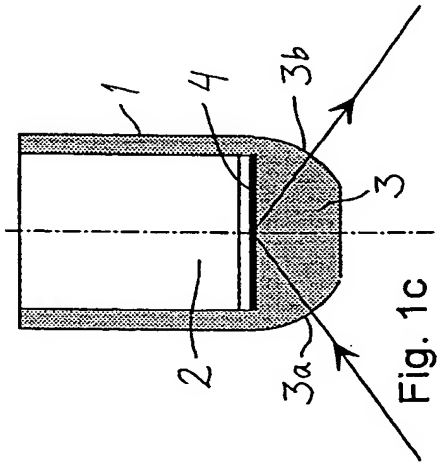

Fig. 1c

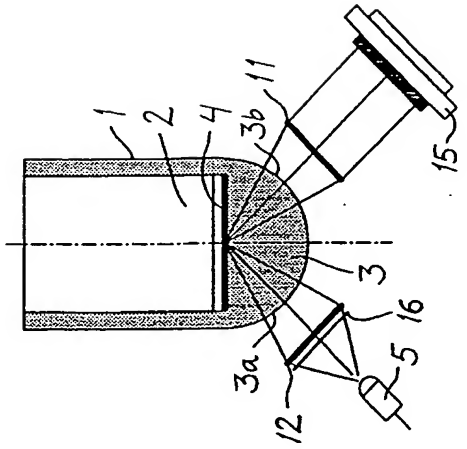

Fig. 2c

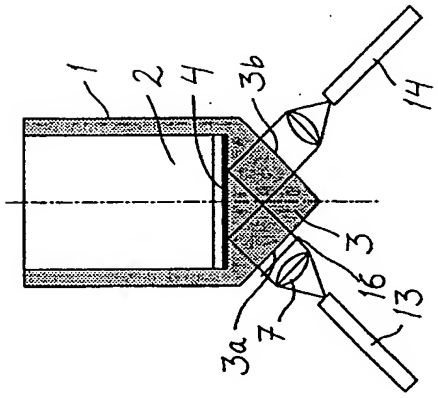

Fig. 2b

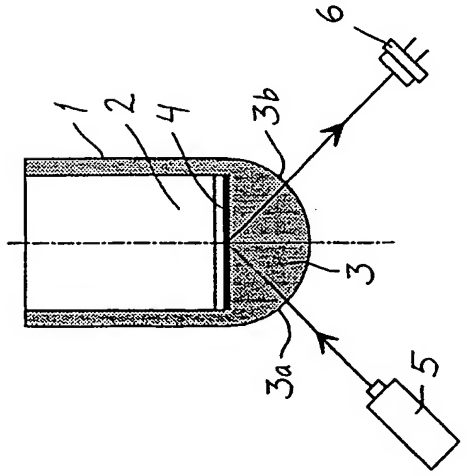

Fig. 2a

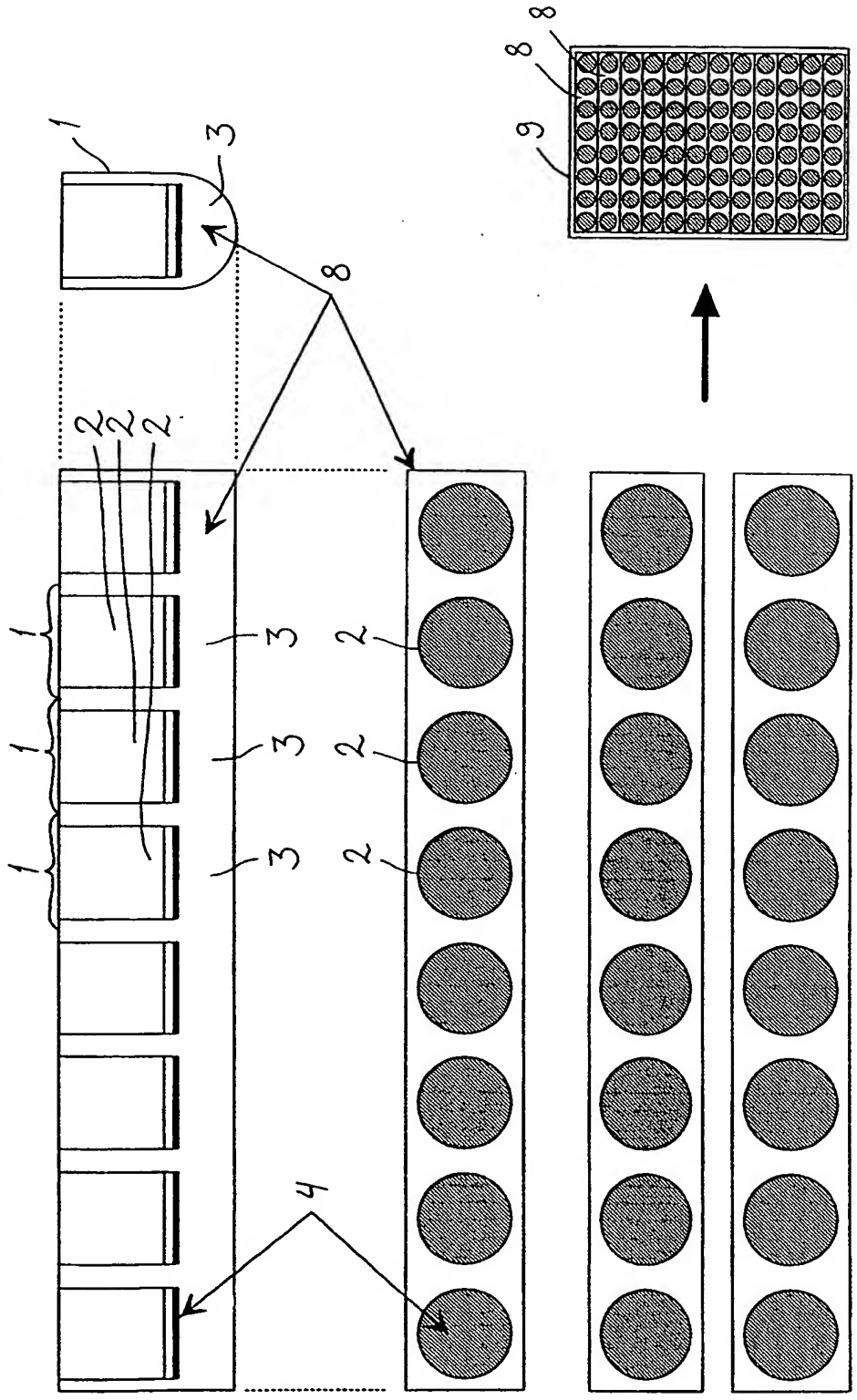

Fig. 3

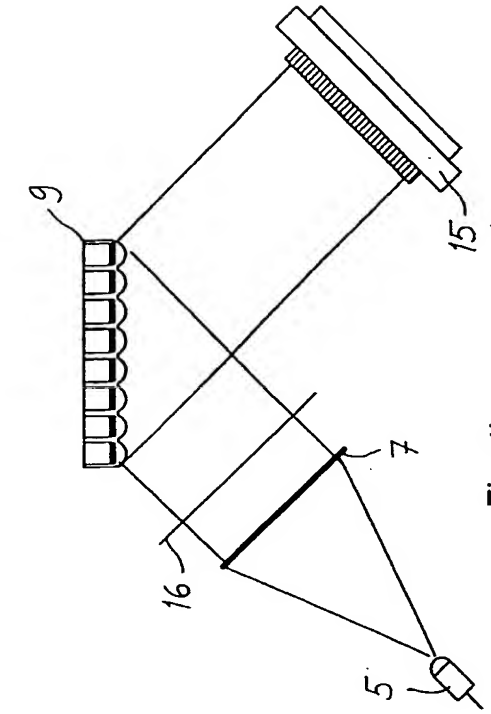

Fig. 4b

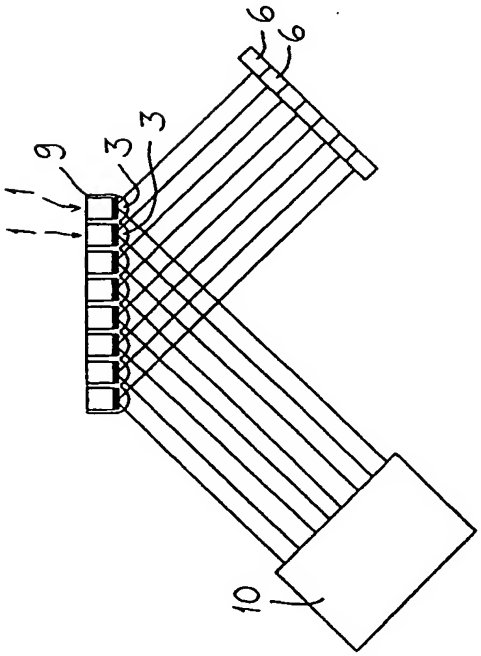

Fig. 4a

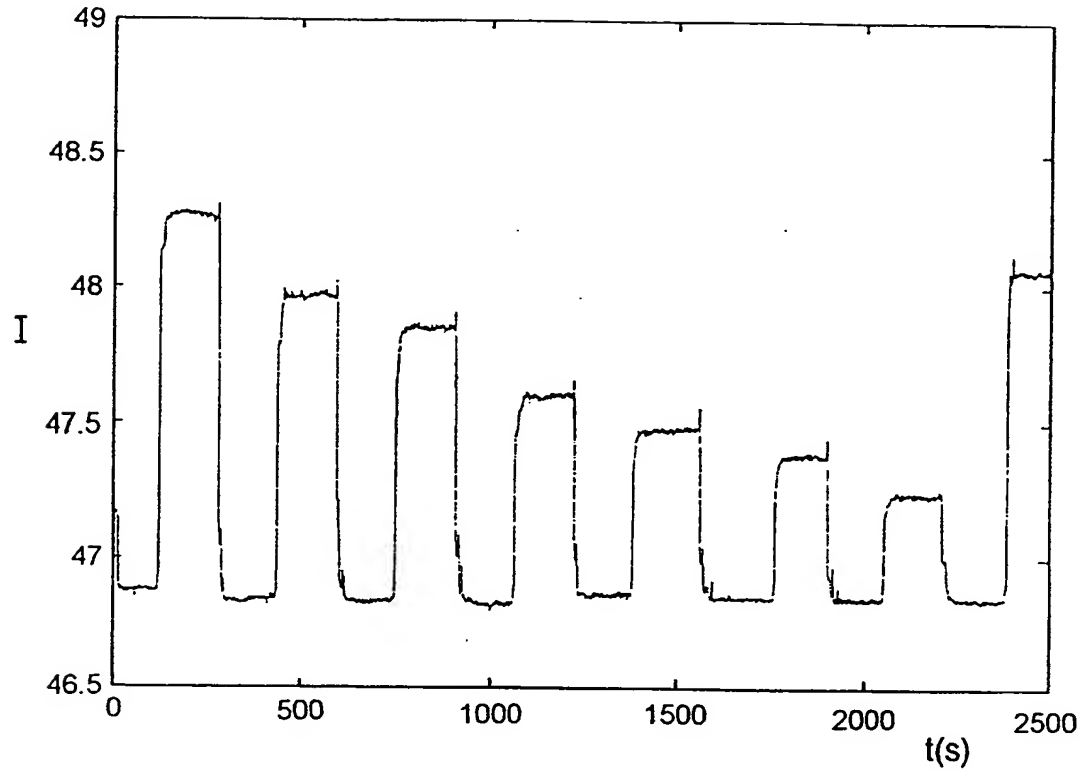

Fig. 5

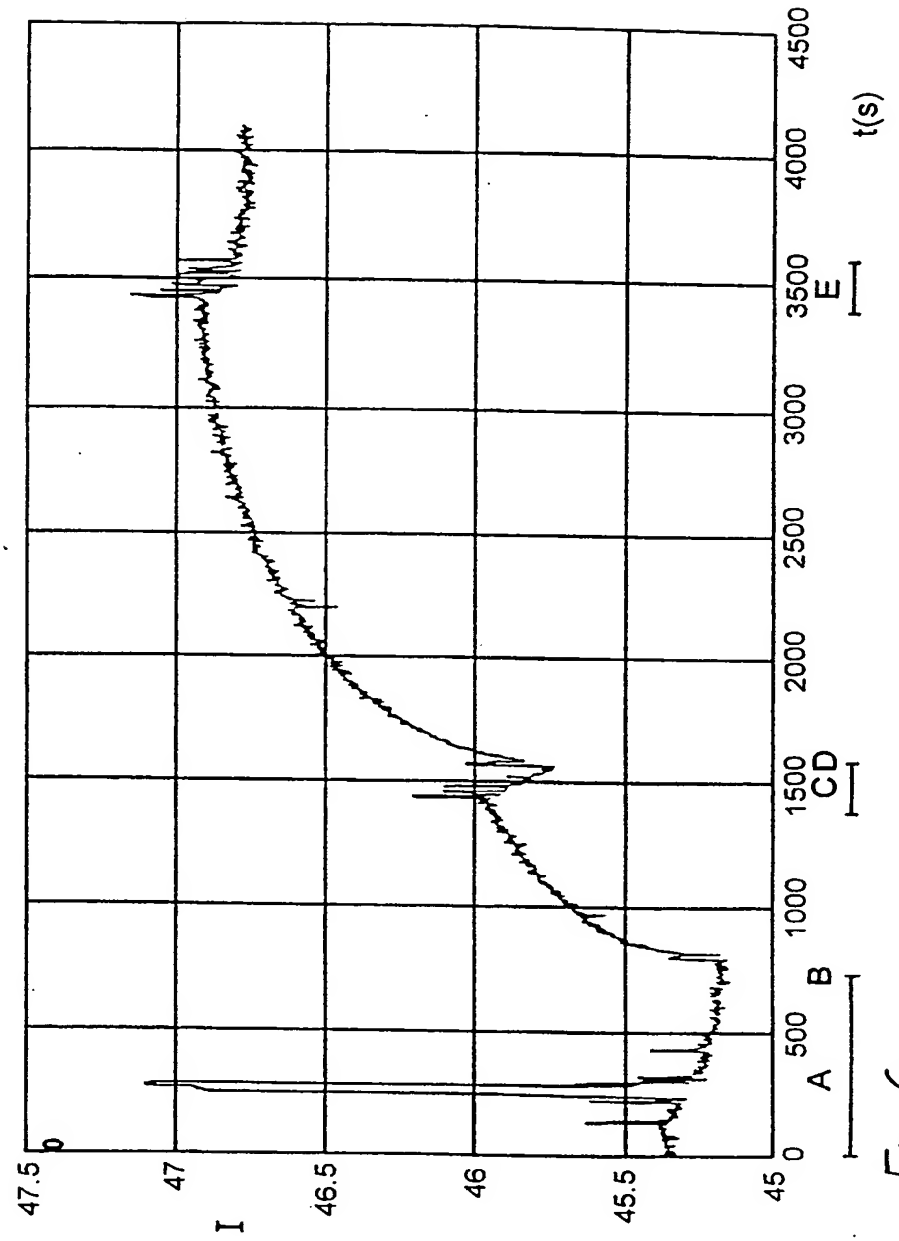
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Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 95/00077

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 21/03, G01N 21/55
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE, A1, 3830002 (NAUMANN, DIETER ET AL), 1 March 1990 (01.03.90), column 2, line 60 - column 3, line 35, figure 1, abstract --	1-5
Y	EP, A1, 0517930 (HEWLETT-PACKARD GMBH), 16 December 1992 (16.12.92), column 14, line 58 - column 16, line 25, figure 5, abstract --	1-5
A	WO, A1, 8200359 (LABSYSTEMS OY), 4 February 1982 (04.02.82), page 6, line 7 - page 7, line 7, figure 2, abstract --	1-5

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 May 1995	09 -06- 1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Karin Säfsten Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 95/00077

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0286195 (NEDERLANDSE ORGANISATIE VOOR TOEGENPAST NATUURWETENSCHAPPELIJK ONDERZOEK TNO), 12 October 1988 (12.10.88), column 3, line 53 - column 4, line 27, figure 1, abstract -- ---------	1-5

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/04/95

International application No.
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